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The 39th EUCHEM Bürgenstock Conference on Stereochemistry

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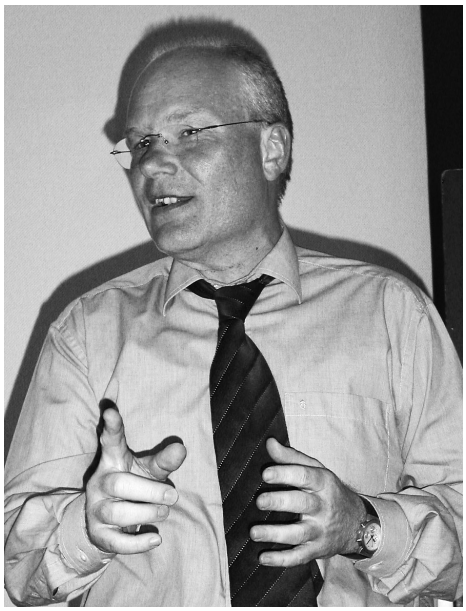
Over 130 scientists, both from industry and academia, from 17 countries worldwide found their way to the Bürgenstock to attend the legendary EUCHEM Conference on Stereochemistry, not only to enjoy a very interdisciplinary scientific program but also to be enchanted by a stunning view of Lake Lucerne and the surrounding mountains. It is this beautiful setting of the Bürgenstock Hotels and Resort that creates the famous relaxed Bürgenstock atmosphere, which sparks numerous discussions about future perspectives in chemistry. The president of this year's conference ***Herbert Waldmann*** (MPI Dortmund, Germany), the vice-president *Alain Krief* (University of Namur, Belgium) together with the local organising committee *Hans-Beat Bürgi* (University of Bern), *François Diederich* (ETH Zürich), *E. Peter Kündig* (University of Geneva), and *Klaus Müller* (Hoffmann-La Roche, Basel), succeeded in attracting 16 excellent speakers, whose names traditionally are kept secret until the first evening.



Herbert Waldmann (President)

Ekkehard Winterfeld (Guest of Honour), Alain Krief (Vice-President), Jan. E. Bäckvall (President 2003)

Moreover, thanks to the generous financial support from the Swiss National Science Foundation and from the Fonds der Chemischen Industrie, the organising committee was able to invite 22 promising young European scientists to attend the conference and present their work and results in poster-form. On the first evening of the conference, which started with an exquisite dinner at the Palace Hotel, President Waldmann warmly welcomed all the participants, and in particular *Ekkehard Winterfeld* (University of Hannover, Germany), the guest of honour of the 2004 Bürgenstock conference.

The first day of the conference was devoted to biochemistry and was opened by *Margaret Kayser* (University of New Brunswick, Canada), who presented the first speaker, ***Roger S. Goody*** (MPI Dortmund, Germany). In his very illustrative talk he introduced the audience to the complex subject of intracellular vesicular transport, which can follow an exocytotic (towards excretion) or an endocytotic pathway (endocytosis).

It is known that this vesicular transport is regulated by Rab proteins, and there are over 60 different such proteins identified to date. As Goody pointed out, Rab proteins are GTPases and can switch between GDP-bound (off-state) and GTP-bound (on-state) forms, which have different conformations. In the GTP-bound form, the Rab GTPases recruit specific sets of effector proteins onto membranes, whereas the GDP-bound form interacts with Rab escort protein

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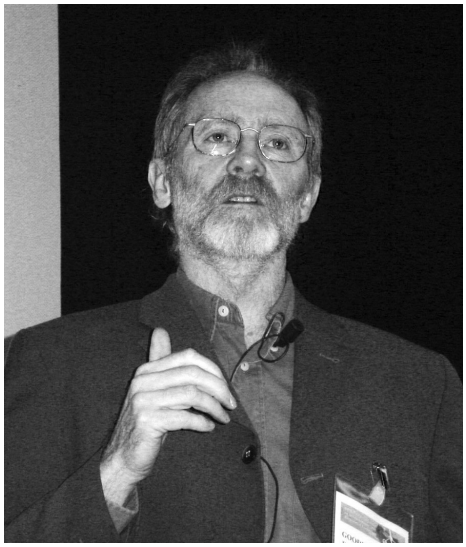


Roger S. Goody

(REP) and GDP dissociation inhibitor (GDI). According to the currently accepted model such REP-Rab complexes are presented to the Rab geranylgeranyl transferase (RabGGTase), which covalently attaches two geranylgeranyl moieties to two cysteine side chains. This post-translational modification is essential to enable the Rab GTPase to associate reversibly with its intracellular target membrane. By means of crystal structures of multi-protein complexes Goody explained mechanistic and structural details of this double-prenylation. His highly interesting talk provoked several questions in the following discussion, for instance: 'How does the prenylated Rab protein know which is its target membrane in the cell?' (a question still as yet unanswered…).

The second morning lecture was given by ***Ilme Schlichting*** (MPI Heidelberg, Germany) about control and reactivity of heme proteins *via* molecular architecture. She pointed out that the reduction potential and

Ilme Schlichting

reactivity of the heme iron, properties which are important for the physiological function of these proteins, are controlled by architectural features such as the proximal ligand coordinating to the iron, the interaction of the propionate groups of the protoporphyrin IX with the surrounding protein matrix, the porphyrin macrocycle geometry and the spin state of the iron.

In the first part of her presentation Schlichting talked about her group's contributions to the field of Cytochrome P450s. In some cases (for example $P450_{BM\text{-}3}$), substrate binding causes a dramatic conformational change (induced fit), thereby enclosing the substrate completely and restricting the access of water molecules. The latter effect is important since water can hydrolyse high-valent iron oxo species formed during the catalytic cycle of the enzyme, which would produce hydrogen peroxide (a process also called uncoupling). In the case of $P450_{cam}$ it is not obvious how the camphor can reach the active site, which is deeply buried in the protein. Using xenon-pressurised single crystals of $P450_{cam}$, the Schlichting group could identify hydrophobic binding sites of this noble gas in the enzyme and hence reveal possible access channels of the hydrophobic substrate camphor. More impressively, the structures of reaction intermediates were determined by initiating reactions in $P450_{cam}$ single crystals using a combination of chemical methods and Laue crystallography with subsequent freeze-trapping of the intermediates. Some of those intermediates are normally very short-lived and for the first time these snapshots of the catalytic cycle of $P450_{cam}$ revealed some very interesting details, *e.g.* the source of the protons which protonate the iron oxygen complex to form the oxidising species. Schlichting stressed, however, that there is still an ongoing debate in the P450 community what exactly the structure of the oxidising species looks like. The second part of her talk was about NO synthases (NOS), which are very similar to P450s and catalyse the two-step reaction of arginine to citrulline and NO. Schlichting discussed possible structures of reaction intermediates on the basis of crystal structures of, *e.g.* the cyanide complex of NOS as an analogue of the oxygen-bound form and complexes of specific inhibitors with different isoforms of NOS.

The first poster session on Sunday afternoon was opened by five short oral presentations (poster session appetisers) by *J. Willem Back* (University of Amsterdam, The Netherlands, 'Bio-Orthogonal Chemistry: Alternatives for Protein Modification'), *Holger F. Bettinger* (University of Bochum, Germany, 'Experimental and Computational Investigations on the Chemistry of Carbon Nanotubes'), *Sergey A. Kozmin* (University of Chigaco, USA, 'Chemical Synthesis: From New Reactivity to Cell-Regulatory Function'), *John E. Moses* (University of Oxford, UK, 'Biomimetic Studies Towards Natural Products'), and *Alexander A. Tishkov* (University of Munich, Germany, 'Structure and Stereodynamics of N,N-Bis(siloxy)enamines').

After dinner, *Chris Abell* (University of Cambridge, UK) was pleased to introduce the speaker of the Sunday evening lecture, ***Michel Rohmer*** (Université Louis Pasteur, Strasbourg, France). He gave an interesting talk about the discovery and elucidation of an overlooked pathway for isoprenoid biosynthesis in bacteria and plants.

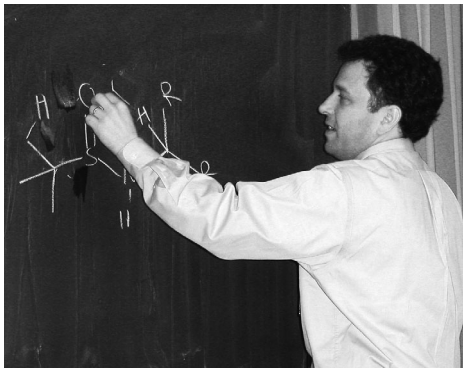
Michel Rohmer

Rohmer highlighted in the introduction that for many years the well-known acetate/mevalonate (MVA) pathway has been unambiguously considered to be the only biosynthetic route to produce isoprenoid C_5 precursors like isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). However, this picture was revealed to be partially incorrect after incorporating experiments with ^{13}C labelled acetate and glucose into bacterial hopanoids did not result in the expected labelling pattern predicted from the MVA pathway. After an impressive amount of devoted work carrying out extensive incorporation experiments using ^{13}C and ^{2}H labelled precursors and subsequent analysis by NMR and mass spectrometry, the group of Rohmer could elucidate this novel methylerythritol phosphate (MEP) pathway and identify key enzymes, intermediates and precursors such as pyruvate, glyceraldehyde 3-phosphate, and desoxyxylulose. As he pointed out, this metabolic route is found in most bacteria as well as in plant chloroplasts. More importantly, the MEP pathway is present in many pathogenic and opportunistic bacteria and protozoa, including for instance the agents

responsible for tuberculosis or malaria, and is absent in humans. Accordingly, all enzymes are potential targets for a novel type of antibacterial or antiparasitic agents. Rohmer closed his presentation with the recent discovery that there might be some interchange or 'communication' between the MVA and the MEP pathway at the level of mono-, di- and sesquiterpenes, which can make it very difficult to track the pathway that a certain precursor is 'travelling' in the biosynthesis of a specific isoprenoid.

Monday morning started with a surprise: the Bürgenstock was covered in white overnight! Thus, the group photo was taken in the snow.

Catalysis and methodology in organic chemistry was the overall topic of the Monday lectures. Last year's president of the Bürgenstock conference, *Jan-E. Bäckvall* (Stockholm University, Sweden), presented ***Jonathan Ellman*** (University of California, Berkeley, USA) as the first speaker, who gave an excellent presentation about new methods for stereoselective carbon–nitrogen and carbon–carbon bond formation.



Jonathan Ellman

In the first part of his talk Ellman focussed on the asymmetric synthesis of amines, which after all are present in over 75% of drugs and drug candidates. The method developed in his research group is based upon the use of enantiomerically pure tert-butanesulfinamide, which is prepared using catalytic enantioselective methods in two steps in 71–75% overall yield from *tert*-butyl disulfide, an extremely inexpensive oil waste byproduct. Direct condensation of the chiral sulfinamides with aldehydes and ketones provides sulfinimines in uniformly high yields which then can react with different nucleophiles, such as Grignard-type reagents, in a highly diastereoselective fashion. The desired chiral amine product is then obtained after the subsequent cleavage of the sulfinyl group with protic acid. Ellman demonstrated the high versatility of this powerful method with a few examples, *e.g.* these chiral ketimines can be reacted with metal enolates and yield highly substituted β-aminoacids after hydrolysis of the auxiliary group. Alternatively, the ketimines can be used to generate metalloenamides, which are reacted with aldehydes. The resulting β-hydroxysulfinimines are then further reduced stereoselectively to yield either optically pure 1,3-*anti* or 1,3-*syn* amino alcohols. This tert-butanesulfinamide methodology was also explored on solid support and applied to access bioactive natural products of the pavine and isopavine classes. The second part of the lecture dealt with the use of heteroatom-directed C–H activation for carbon–carbon bond formation. The Rh-catalysed annulation reaction of alkenyl substituted aromatic amines and heterocycles, such as pyridines and benzimidazoles, allowed the Ellman group to prepare multicyclic compounds in one step. The highly practical sulfinimine methodology in particular provoked many interesting questions from industrial researchers in the following discussion.

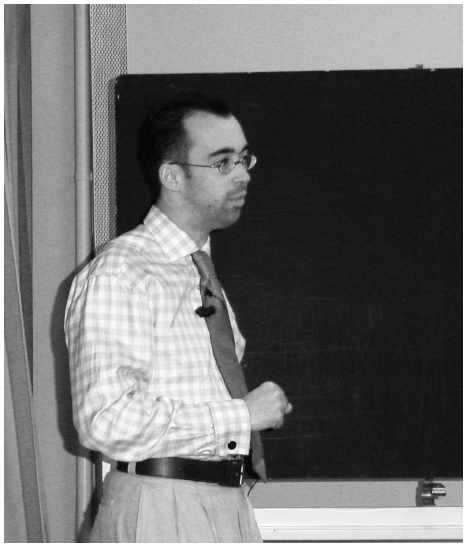
The second morning talk was given by ***Matthias Beller*** (University of Rostock, Germany) who addressed the issues of carbon–carbon bond formation, hydroamination, multi-component reactions (MCR), and amidocarbonylation under homogeneous catalysis. Even though amino acids have become accessible by new – especially enantioselective – routes in the last few years, classical methods for their synthesis like the Strecker reaction are still used in technical processes.

Matthias Beller

As it became clear in Beller's presentation, his group is interested in the development of new, particularly technically feasible methods for the synthesis of amino acids. Certainly their Pd-catalysed multicomponent coupling of aldehydes and amides in the presence of carbon monoxide (amidocarbonylation) is intriguing, which efficiently provides N-acyl amino acids without concomitant formation of stoichiometric amounts of byproducts. The use of palladium as active metal allows the catalytic amidocarbonylation of a variety of aldehydes under mild reaction conditions. During the course of optimising the multi-component amidocarbonylation reaction, formation of amidodienes was observed when using very low catalyst loadings (<1:10'000), and by adding dienophiles to the reaction those amidodienes could be trapped to yield cyclohexenylamides. Using this four-component catalytic approach the Beller group was able to establish a library of 200 members, some of them being promising precursors of biologically and pharmaceutically active compounds. Beller then switched to another project in his group, the catalytic functionalisation of aryl halides, especially chloroarenes and similar compounds, to obtain aromatic amines, arylated olefins, benzaldehydes, benzoic acid derivatives, and benzonitriles, all of which are of enormous importance for the chemical industry as pharmaceuticals, agrochemicals, and fine chemicals. In this regard he presented some of the recent catalyst systems developed in his group, which are based on adamantyl, N-phenylpyrrole, and N-phenylindole phosphines. Very interesting was the part about catalytic cyanations of aryl halides to prepare benzonitriles. The problem of catalyst deactivation by cyanide coordination was elegantly circumvented by continuous addition of defined amounts of cyanide, *i.e.* by using the cyanohydrin of acetone or $K_4[Fe(CN)_6]$ in the presence of sodium carbonate in the reaction mixture. Finally, in the last part of the talk Beller presented some recent developments from his laboratory in the field of efficient synthesis of organic bulk chemicals from cheap bulk olefins or olefinic mixtures by using Pd-catalysed telomerisations, Rh-catalysed hydroformylations and metal-catalysed hydroaminomethylations.

Another tradition of the Bürgenstock conference is that the afternoons are free for recreation, such as going for hikes in this marvellous region, informal discussions, poster sessions, and also a special session which took place on Monday afternoon and was chaired by *E. Peter Kündig* (University of Geneva). ***Nicholas E. Leadbeater*** (University of Connecticut, USA) was the first speaker of this special session and the topic of his talk was metal-mediated synthesis without the metals or as he put it 'from organometallic to orgaNOmetallic'.

There is barely a modern kitchen without a microwave oven and Leadbeater convinced the audience that the same could soon be true of chemistry laboratories. Nowadays there exist scientific microwave ovens that allow accurate control of temper-



Nicholas E. Leadbeater

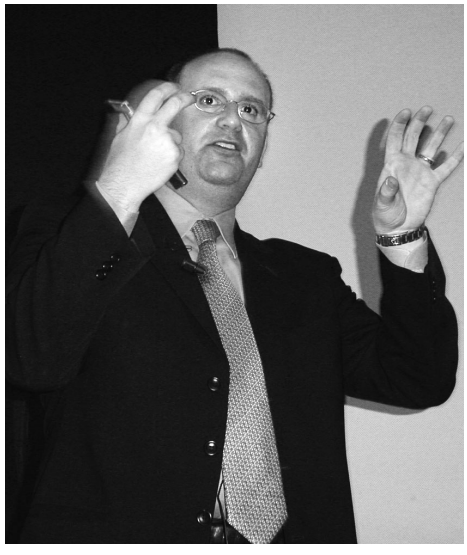
Milton R. Smith III

ature, microwave power and pressure, which is essential for reproducibility of results. Microwaves heat reactants much more quickly than conventional means and therefore can reduce reaction times and enhance yields. Using microwaves, and after some optimisation of conditions, the Leadbeater group elaborated efficient protocols for Ni-catalysed halogen exchange or cyanation of aryl halides. Water is the solvent of choice in microwave synthesis, which opens the door to green chemistry. Leadbeater presented examples of microwave Suzuki reactions in water, and most astonishingly this C–C coupling also worked when the palladium was omitted! Later they also found successful examples of metal-free Sonogashira and Heck coupling reactions in water. Not surprisingly, the proposed mechanism of these unusual metal-free microwave reactions sparked an interesting debate after his talk.

The second speaker of the special session was ***Milton R. Smith III*** (Michigan State University, USA). His group is involved in the research area of transition metal chemistry of boron. During the course of exploring the fundamental chemistry of metal boryl complexes (M-BX_2) and examining reactions of boron-element bonds with unsaturated organic ligands coordinated to metal centres they eventually discovered unusual selectivities for olefin borylation reactions.

Smith presented an extension of this chemistry with the recently reported catalytic pinacolborylation of benzene using iridium catalysts like Cp*Ir(PMe_3)(H)(BPin) or Cp*Ir(PMe_3)$(H)_2$. This certainly very useful reaction marks the first example of the catalytic synthesis of a B–C bond from an arene C–H bond and a borane B–H bond. Significantly, if this reaction was carried out with aryl mono-fluorides, -chlorides, -bromides or -iodides, activation of the halide did not occur and mixtures of *meta*- and *para*-borylated aryl halides were obtained instead. Smith also showed some examples of pinacolborylation of heterocycles such as thiophene, N-protected pyrrole, and indole. A unique feature of the catalytic chemistry developed in his laboratory is the influence of steric effects on regioselectivity. 1,3-substituted benzene rings are selectively borylated at the 5-position, which is typically the least activated site towards aromatic substitution. The boron atom of such products can subsequently be transformed into an OH group in the same pot by treatment with oxone and consequently provide 1,3,5-substituted phenols in an elegant manner.

It was *Johann Mulzer* (University of Vienna, Austria) who introduced the speaker of the Monday evening lecture, ***Matthew D. Shair*** (Harvard University, USA), whose group is strongly interested in biomimetic synthesis applied to chemistry and biology.



Matthew D. Shair

This also includes biomimetic target-oriented synthesis of complex naturally occurring molecules and Shair exemplified this concept with their recently accomplished first total synthesis of (–)-longithorone A.

This cytotoxic marine natural product with an unusual heptacyclic structure contains two forms of chirality: stereogenic centres and atropisomerism arising from the hindered rotation of a quinone ring through a macrocycle. In their retrosynthetic design the Shair group followed a provocative hypothesis to explain the biosynthesis of (–)-longithorone A involving an intermolecular Diels-Alder cycloaddition between two [12]-paracyclophane precursors followed by a transannular Diels-Alder reaction across one paracyclophane unit to simultaneously assemble three rings. The syntheses of two similar [12]-paracyclophanes were realised by using ene-yne metathesis macrocyclisation, which is the first reported example of this kind of reaction. The Lewis acid-catalysed intermolecular Diels-Alder reaction between the two [12]-paracyclophanes was not diastereoselective, however, which led to the suggestion that a Diels-Alderase may be involved in this step in the biosynthesis of (–)-longithorone A. On the other hand, the following transannular Diels-Alder reaction occurred at room temperature completely regio- and stereoselectively, and directly afforded the target compound. Not only did Shair and his co-workers find strong evidence for the proposed biosynthesis by the completion of their biomimetic total synthesis of (–)-longithorone A, but also demonstrated in a most impressive way how chirality can be transferred by use of stereogenic centres to control atropisomerism and then transfer of the atropisomerism back to stereogenic centres in the natural product. The last part of his talk concerned decarboxylative aldol additions of β-carboxythioesters. This particular *in situ* enolisation and subsequent reaction with an electrophile was inspired by a similar step in polyketide biosynthesis and, as Shair illustrated with a range of examples, was successfully applied in his laboratory.

Tuesday was the day of material science and both morning lectures demonstrated very powerfully that synthetic (organic) chemists can make important contributions to this area. The first speaker introduced by *Ben L. Feringa* (University of Groningen, The Netherlands) was ***Atsuhiro Osuka*** (Kyoto University, Japan). In his colourful talk he highlighted how one can use simple metal porphyrins as monomeric building blocks and link them together to produce huge linear nanostructures with extremely interesting physical properties.

It was discovered that the nitration of 5,15-diphenyl zinc porphyrins using Ag-

Atsuhiro Osuka

NO_2 and I_2 produces, along with the nitrated product, the *meso-meso* linked porphyrin dimer in 10% yield. When treating 5,15-diaryl zinc porphyrins with a silver salt having a less nucleophilic anion, like SbF_6, dimer and even trimer formation was observed. The Osuka group was able to determine the crystal structure of the trimer, and intriguingly the individual porphyrin planes are twisted against each other with a dihedral angle of almost exactly 90°. This structural feature is very important for the solubility of these *meso-meso* linked porphyrin oligomers in organic solvents. Osuka presented to the astonished audience how they had pushed this silver-mediated dimerisation chemistry to the limit by synthesising a *meso-meso* linked zinc porphyrin 1024mer! This linear nanostructure has an impressive length of 0.86 μm and could readily be seen under the electron microscope. Later his group also found conditions to produce linear *meso*-β linked porphyrin arrays with a completely different macroscopic structure. In addition, Osuka showed how the twisted *meso-meso* linked porphyrin oligomers can be transformed into planar *meso*,β,β-linked systems using $Sc(OTf)_3$/DDQ. Such extended π-conjugated planar superstructures have very interesting optical properties, *e.g.* the so-called porphyrin Q-band of the higher *meso*,β,β-linked porphyrin oligomers absorbs already in the near-IR region. Nature uses circular porphyrin arrays as light-harvesting systems, and inspired by this the Osuka group carried out the silver-mediated dimerisation reaction under high-dilution conditions with a linear *meso-meso* linked porphyrin 12mer. The obtained cyclic *meso-meso* linked porphyrin 12mer could also be visualised by electron microscopy and has an impressive diameter of ~35 Å. The last part of Osuka's lecture was about expanded porphyrins, which can be prepared as a mixture by using fluorinated benzaldehydes, pyrrole, and specific porphyrin condensation conditions and later separated. Such expanded porphyrins can have a multitude of oxidation states, undergo facile $2e^-$ oxidations and reductions and act as multi-metal chelates. Osuka illustrated how they bound Cu^{2+} ions to a free base octaphyrin and experienced an amazing surprise: upon standing the *bis*-copper octaphyrin split into two copper porphyrins! This molecular mitosis, as he called it, a beautiful and at the same time bizarre spontaneous reaction, left the audience in astounded excitement.

Every good electrician knows that an electrical wire needs to have an insulation but how do you insulate a molecular wire? An inventive answer to this question was given by ***Harry L. Anderson*** (University of Oxford, UK), who was the second speaker of the morning session. Conjugated and therefore conducting organic polymers such as poly(*para*-phenylene) have been used to prepare light-emitting diodes (LEDs). However, solid-state packing effects between the polymer strands lead to partial quenching of luminescence and shift light emission to a broader and longer wavelength (red shift). Blue in particular is the hardest colour to achieve.

Harry L. Anderson

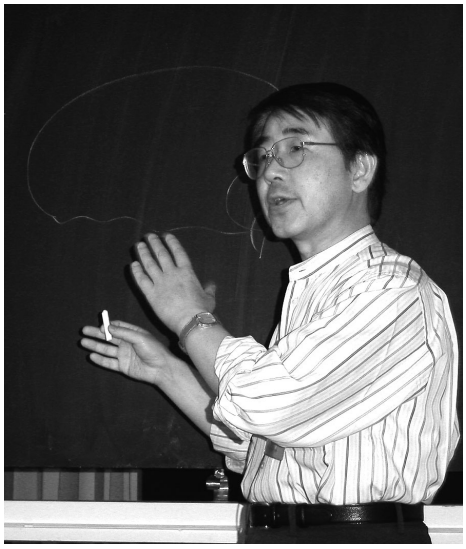
In his talk Anderson sketched out his approach to use conjugated polyrotaxanes as a supramolecular insulated wire. From NMR experiments in D_2O the Anderson group learned that due to the hydrophobic effect, lipophilic polymer building blocks such as 1,4-dipropynebenzene could thread through water-soluble cyclophanes. Using this particular host–guest interaction such threaded monomers were coupled under Cu^+-catalysis in water to form sheeted conjugated polymers, and then capped with bulky stoppers in order to prevent unthreading. Using this strategy the laboratory of Anderson achieved the synthesis of compounds with a remarkable supramolecular architecture and indeed, they could demonstrate that insulation enhances the stability and luminescence of the molecular wire while its semiconductivity is preserved. Moreover, they showed that the LEDs made from these wires are more efficient than those made from analogous uninsulated conjugated polymers. This concept of encapsulation was also applied to synthetic dyes, which enrich many aspects of everyday life and have high-tech applications in opto-electronics. As Anderson illustrated in his talk, his group used α- or β-cyclodextrins (CDs) to encapsulate dumbbell shaped fluorescent chromophores. By doing so the dye is protected from the environment and in addition its properties are modified in a variety of ways. For instance, the photoisomerisation of α-CD encapsulated *trans*-stilbene type dyes is dramatically retarded. At the end of his lecture Anderson also showed some examples where cyclodextrins instead of cyclophanes have been used to encapsulate molecular wires, and again he pointed at the benefits of encapsulation towards the prevention of aggregation, reduction of ground state reactivity, enhancement of photostability, and reversible electrochemistry.

After three days of excellent lectures, poster and special sessions and plenty of scientific discussions it was time for some muse. After a delicious dinner at the Bürgenstock 'Le Club', the *Aura String Quartet* from Basel (Antonio Núñez, Roger Pyne, Christian Vaucher, and Conrad Wyss) enchanted the conference participants with some fine chamber music. Their stunning performance of pieces from Johann Sebastian Bach, Dimitri Shostakovich and Bedrich Smetana left a visibly moved president, who invited everyone to a wine reception afterwards resulting in relaxed late-night discussions.

Aura String Quartet, Herbert Waldmann

Wednesday focussed on biology and bioorganic chemistry, and was chaired in the morning by *Christof Niemeyer* (University of Dortmund, Germany), who an-

nounced the speaker of the first lecture, ***Kazunari Taira*** (University of Tokyo, Japan). He started by introducing the audience to the topic of ribozymes, which are RNA molecules with enzymatic activity and were discovered about 20 years ago. Ribozymes, including hammerhead ribozymes (HHR, because of the resemblance of their two-dimensional structure to a hammerhead) are actually metalloenzymes and use Mg^{2+} to catalyse the sequence-specific cleavage of abnormal mRNA.



Kazunari Taira

Taira presented the contributions of his group in order to clarify the exact mechanism of this phosphodiester bond cleavage reaction using kinetic isotope effect measurements among other methods. In the past the use of ribozymes such as HHR to elucidate and eliminate gene functions has been attempted. But as Taira stressed, ribozyme activity *in vivo* critically depends on a number of technical issues such as the effective level of expression, specificity, intracellular stability, target co-localisation, and accessibility to the target site. Over the years his group has successfully explored the use of dimeric minizymes as gene-inactivating agents by placing minizymes under the control of a human $tRNA^{Val}$ promoter. With their powerful ribozyme expression system they were able to increase the *in vivo* cleavage activity of this minizyme substantially. Most impressively, by linking a helicase-binding motif to a ribozyme that recruits helicase protein and its unwinding activity, the Taira group could overcome the problem of target sequence inaccessibility. Such helicase-coupled ribozymes were indeed shown to have substrate-unwinding as well as enhanced cleavage activity in *in vitro* and *in vivo* assays. As an extension of the molecular design based on the heterodimeric form of short ribozymes, Taira explained how they have constructed an allosterically controllable novel enzyme (designated maxizyme), which has sensor arms that can recognise target sequences. In the presence of only such a target sequence, it can form a cavity that can capture catalytically indispensable Mg^{2+} ions. Very interestingly, when targeting *BCR-ABL* mRNA, the maxizyme induced apoptosis only in leukemic cells without affecting normal cells and thus underlined that the maxizyme is particularly valuable as a gene-inactivating agent with therapeutic potential. Taira also illustrated how libraries of hybrid ribozymes can be utilised in order to discover specific genes. Introduction of such libraries into HeLa-Fas cells using a retrovirus expression system was combined with a selection of surviving clones, which did not undergo apoptosis. Isolation of ribozymes from the selected cells followed by sequence and database analysis led to the identification of the target genes of ribozymes which have a pro-apoptotic function. The Taira group applied the same gene discovery technique to identify genes, which are responsible for cancer cell metastasis and interestingly many of these genes lie in the so-called 'junk' region of DNA. Taira is convinced that many of the target sequences were ignored and forgotten because they did not belong to the coding genome. In particular, recently discovered small RNAs coming from this non-coding region such as microRNAs (miRNAs) and short interfering RNAs (siRNAs) have emerged as key components of an evolutionarily conserved system of RNA-based gene regulation in eukaryotes and is just beginning to be understood. It became apparent from Taira's comprehensive lecture that there are still plenty of undiscovered places in the RNA world.

The second lecture on Wednesday morning also dealt with ribozymes and ***Michael Famulok*** (University of Bonn, Germany) emphasised how these interesting small RNA molecules could be utilised in drug screening. *In vitro* selection of combinatorial nucleic acid libraries (abbreviated as SELEX) leads to specific target-binding molecules like RNA, ssDNA, modified RNA or modified DNA, commonly designated as aptamers, and to novel catalytic nucleic acids (ribozymes).

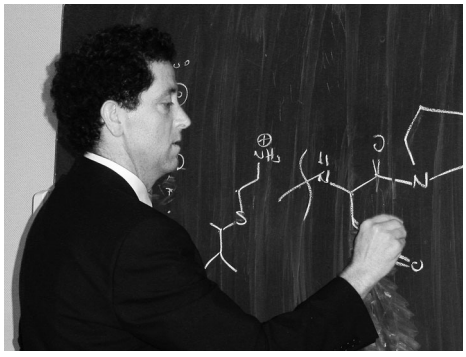
Michael Famulok

As Famulok pointed out it is possible nowadays to obtain aptamers for a large variety of targets such as small organic molecules, amino acids, biological cofactors, peptides, and proteins by SELEX technology. He also illustrated how his group is using these aptamers very elegantly as tools in molecular biology. Intramers are aptamers which bind to intracellular targets, and the Famulok group developed a virus-based RNA expression system enabling high-level cytoplasmic expression of RNA aptamers directed against cytohesin 1, which plays an important role in the regulation of cell adhesion in leukocytes. Their designed aptamer could discriminate between the latter protein and cytohesin 2 (also called ARNO), which is very similar. One of the conclusions they could draw from cell experiments with this aptamer was that in non-immune cells the Serum-Response-Element transcription (c-Jun/c-Fos pathway) is controlled by ARNO rather than cytohesin 1. These results demonstrated that their concept of aptamer-controlled blocking of signalling pathways *in vivo* in order to reveal important details from these complex processes proves to be very efficient, and as Famulok mentioned could potentially be applied wherever targeted modulation of signal-transduction cascades is desired. Another potential application of aptamers, which he described in his talk was using reporter ribozymes for high-throughput drug screening. This novel assay is based on a FRET-oligonucleotide (FRET = fluorescence resonance energy transfer), which forms a hybrid with a ribozyme/aptamer chimera. A specific target protein of interest can bind to the aptamer domain and in the FRET-substrate the fluorescence of a fluorophore, which is in close spatial proximity of a fluorescence-quenching molecule, is intramolecularly quenched. In the presence of a drug-like molecule with a high affinity towards the bound protein of interest the latter can then be displaced and the resulting conformational change and ribozyme activity cleaves the FRET-substrate. As a result the fluorophore is liberated and a fluorescence signal is generated which can be quantified by an appropriate read-out system in real-time. Certainly, a very clever and intriguing concept, and Famulok's excellent presentation made it clear that designed small RNA molecules have a great future.

The afternoon was reserved for the second part of the poster session and was again

opened by short oral presentations: by *Rolf Breinbauer* (MPI Dortmund, Germany, 'Electrons as a Reagent in Solid Phase Synthesis'), *Fabien Gagosz* (DCSO-Ecole Polytechnique, Palaiseau, France, 'Nitrogen Centered Radicals. Useful Intermediates for the Synthesis of Nitrogen Containing Heterocycles'), *Christopher J. Hayes* (University of Nottingham, UK, 'Vinylphosphonate-linked Oligonucleic Acids: Stereoselective Synthesis and Use as Biological Probes'), *Bernd Plietker* (University of Dortmund, Germany, 'The RuO_4-Catalysed Ketohydroxylation of Olefins'), and *Cordelia Schiene-Fischer* (MPI Halle, Germany, 'Beyond Molecular Chaperone Function: Biocatalysis of Bond Rotations').

Finally, to round up a day full of interesting biology and bioorganic chemistry, *Stefan Matile* (University of Geneva) was pleased to present ***Kevan Shokat*** (University of California, San Francisco, USA) as the evening speaker.



Kevan Shokat

In his lecture he first focussed on the elucidation of protein kinase signalling networks. Undoubtedly a very important but at the same time very challenging task because there are over 500 human kinases known. Moreover, due to their extremely conserved ATP binding pocket, protein kinases have proved to be largely resistant to the design of highly specific inhibitors and the lack of these compounds has complicated efforts to assign specific signalling roles to individual kinases. To distinguish the substrates of one kinase from all other kinase substrates, the Shokat group used a combination of structure-based design and site-directed mutagenesis to make the kinase of interest catalyse a unique phosphorylation reaction not catalysed by any other protein kinase in the cell. Shokat illustrated this elegant chemical genetic strategy with the example of v-Src. The ATP binding site of this specific protein kinase was engineered by single-point mutation of the so-called gatekeeper residue to uniquely accept N_6-benzyl ATP as the phosphodonor substrate. This unnatural ATP analogue is not accepted as a phosphodonor substrate by any other protein kinase and is thus orthogonal to all kinases except the engineered one. To date, this method has been applied to the study of over 20 protein kinases allowing for the discovery of their direct substrates in the presence of all other cellular kinases. Similarly, a functionally silent active site mutation was utilised by Shokat's group to sensitise a target kinase to inhibition by a small molecule that does not inhibit wild-type kinases. As a result the generation of specific inhibitors of many diverse protein kinases has allowed the discovery of fundamentally new roles of kinases in transcription, cell cycle regulation, cell-fate determination and oncogenic transformation. Not only are kinase substrates essential to map kinase cascades but the phosphorylation site identification of a single protein is of tremendous importance as well. Usually proteases are used to digest the phosphoprotein that generates smaller peptide fragments for sequencing. Unfortunately, no natural protease is known which selectively recognises a phosphorylated amino acid and cleaves its substrate specifically at the site of phosphorylation. To solve this problem the Shokat laboratory developed an approach, which relies on the well-established β-elimination of phosphoserine residues to yield dehydroalanine under basic conditions. The subsequent Michael reaction with cysteamine produces an aminoethylcysteine residue which is isosteric with lysine and recognised by proteases like trypsin, Lys-C, and lysyl endopeptidase. Taken together the lecture of Shokat demonstrated in an impressive way that small molecule-based methods can have a major impact in understanding complex molecular biological processes.

The conference was slowly coming to its end and the two lectures of the last morning session were in the context of synthetic organic chemistry. The chairman, *Peter Seeberger* (ETH Zürich, Switzerland), announced ***Henk Hiemstra*** (University of Amsterdam, The Netherlands) who gave a talk on studies towards the total synthesis of solanoeclepin A. This complex natural product is excreted in minute quantities by the potato root and is the most active natural hatching agent of the potato cyst nematode which are organisms causing severe crop losses in potato production.

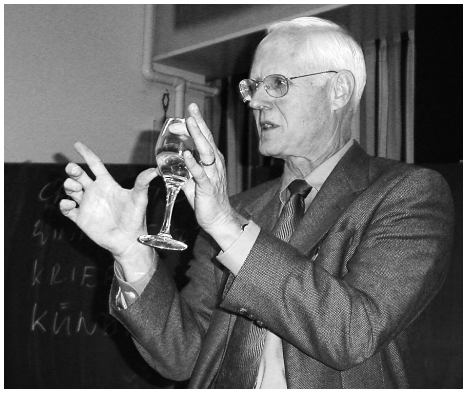
Structure elucidation of solanoeclepin A brought a heptacyclic molecule to light containing all the ring sizes ranging from three to seven, including a highly strained bicyclo[2.1.1]hexanone unit. Hiemstra pointed out that the latter is an unprecedented structural feature in natural products, hence there was also no chemistry known to install such a highly strained moiety when he and his coworkers set off to work out a total synthesis of solanoeclepin A. They were not only intrigued by the beautifully complex structure,

Henk Hiemstra

but also that the target plays a potential role in the search for an environmentally benign method to combat the nematode. Interestingly, to a certain extent the structure of solanoeclepin A resembles that of glycinoeclepin A, the hatching agent of the soybean cyst nematode. After presenting the retrosynthetic analysis Hiemstra highlighted their key step based on a [2 + 2] photocycloaddition to install the bicyclo[2.2.1]hexanone substructure in the target molecule. He described their initial investigation of diastereoselective [2 + 2] photocycloadditions between a 1,3-dioxin-4-one and variously substituted alkenes connected at C(5) with a two carbon tether, in order to arrive at substituted bicyclo[2.2.1]hexanes. This cyclisation process turned out to be remarkably dependent on the substitution pattern of the pendant alkenes, leading either to bicyclo[2.2.1]hexanes (crossed adducts) or bicyclo[2.2.0]hexanes (straight adducts). The former outcome follows the so-called empirical rule of five, which explains the regiochemistry by the supposedly preferential 1,5-closure during the first step of the cyclisation process. Seemingly, di- and trisubstituted alkenes have appeared reluctant to give 1,5-closure and therefore crossed adducts. On the contrary, all of these photocycloadditions proceeded with complete regioselectivity for 1,6-closure yielding straight adducts. Not surprisingly many of these highly strained products were rather unstable and therefore difficult to purify and fully characterise. Thankfully, most of them were crystalline and the stereochemical outcome of the photocycloadditions could be elegantly established on the basis of X-ray crystal structures. Some of the model compounds synthesised, which represent substructures of solanoeclepin A, displayed moderate hatching activity and this gave some idea which part of the molecule is important for the biological activity. Hiemstra's lecture was a marvellous demonstration that to the left and right of an intricate synthetic path towards a complex target there

might be some very interesting chemistry hidden and it is worthwhile to go and explore it.

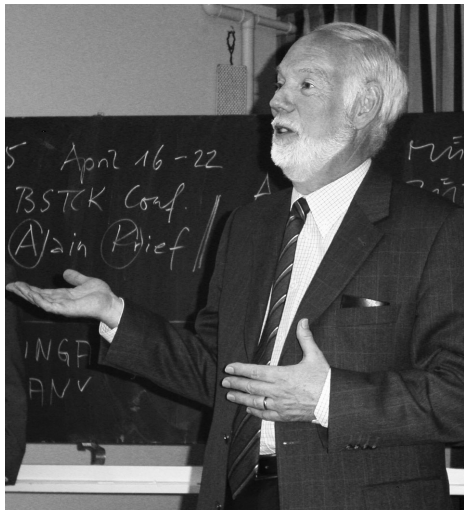
A different methodology to construct all-carbon quaternary centres in a stereoselective and catalytic fashion, still a somewhat 'holy grail' in synthetic organic chemistry, was presented by ***Larry E. Overman*** (University of California, Irvine, USA).



Larry E. Overman

First he introduced the audience to the remarkable group of cyclotryptamine alkaloids that link together up to eight pyrrolidinoindoline units and have been isolated from higher plants. The simplest representative chimonanthine features two vicinal benzylic quaternary centres. In higher order members of this group, exemplified by idiosperminine and quadrigemine C, additional pyrrolidinoindoline units are attached at their benzylic quaternary stereocentres to *peri* positions of the aromatic ring of other pyrrolidinoindoline fragments. Stimulated by the unusual structures and varied biological activities of higher-order polypyrrolidinoindoline alkaloids, Overman and his co-workers proceeded to develop chemistry to access these complex, configurationally diverse alkaloids by stereocontrolled synthesis. As he emphasised the most powerful extant and in his group most established method for enantioselective construction of stereogenic all-carbon quaternary centres is the catalytic asymmetric intramolecular Heck reaction. A particular focus of their investigation in this area has been the enantioselective synthesis of chiral 3,3-disubstituted oxindoles. Starting from 2'-triflato-(*Z*)-2-aryl-2-butenanilides, 3-alkyl-3-aryloxindoles were obtained in high enantiopurity; the wide variety of aryl and heteroaryl substituents, including ones of considerable steric bulk, that can be introduced into an oxindole in this manner is remarkable. Using maleic acid-type bisanilides as substrates the intramolecular double Heck cyclisation stereoselectively furnished bisspirooxindoles, which could further be used as valuable intermediates in total syntheses of pyrrolidinoindole alkaloids. The same intermediate can also be accessed by dialkylation of a metal dienolate of dihydroisoindigo with a tartrate-derived dielectrophile. Interestingly, nonchelating conditions (NaHMDS, THF) gave a single pentacyclic product having a *cis* relationship between the two spirooxindole groups whereas chelating conditions (LiHMDS, THF/DMPU) produced only one of the two possible *trans*-fused C_2-symmetrical bisspirooxindoles. In the last part of his talk Overman highlighted the application of their methodologies in the total syntheses of idiospermuline, hodkinsine and hodkinsine B.

Karl Wieghardt (MPI Mühlheim an der Ruhr, Germany) had the privilege to give the last lecture of the conference, which was chaired by the vice-president *Alain Krief* (University of Namur, Belgium). Wieghardt started his excellent lecture pointing out the fact that essential and trace elements like V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo and W make up about 100 mg per human being. In addition, around 40% of all known enzymes are metalloproteins and in the past six years, among other metalloenzymes, the Wieghardt group has focussed on the copper-containing galactose oxidase (GO) which catalyses the $2e^-$ aerobic oxidation of primary alcohols to aldehydes and hydrogen peroxide.



Karl Wieghardt

GO is structurally well characterised and in the resting state a single Cu^{II} ion is coordinated in a distorted square-based pyramidal fashion to two histidine N-donor atoms, two tyrosinato oxygen atoms and a labile water molecule. One of the tyrosines exhibits a peculiar post-translational modification. It forms a thioether linkage in the *ortho* position to a neighbouring cysteine residue and this *o*-thioetherphenolato moiety is also the locus where one-electron oxidation generates the active form of the enzyme, namely a coordinated Cu^{II}-tyrosyl radical species. As Wieghardt further emphasised this active species is EPR-silent and it has been proposed that the unpaired electron of the Cu^{II} ion is intramolecularly antiferromagnetically coupled to the unpaired electron of the tyrosyl radical, which is rather surprising. He then presented some active site model compounds which have been synthesised and spectroscopically characterised in his group in order to mimic the reactivity of GO and to find an explanation for the unusual mechanism of exchange coupling of the unpaired electrons in GO. Some of the first model compounds studied were based on Cu^{II}-phenolate complexes, where the coordinated phenolate ligand was connected to the coordinated macrocycle 1,4,7-triaazacyclononane. The electrochemistry, EPR- and UV-spectra of these Cu^{II}-phenolate complexes were very similar to GO, however, most of them were sluggish catalysts. Later, Wieghardt and his co-workers eventually found a new class of copper complexes containing two O,N-coordinated *o*-iminobenzosemiquinonato ligands. Some of these compounds did catalyse the aerobic oxidation of benzyl alcohol to benzaldehyde quite efficiently. Wieghardt then turned to another topic, which his research group is involved in, namely the mimicking of the light-driven water-oxidising Mn_4-cluster in photosystem II. Due to the low resolution of the solved X-ray crystal structure of photosystem II it is not clear what exactly this essential cluster looks like. It became apparent from Wieghardt's talk that despite a vast amount of spectral data, mainly from EPR and EXAFS analysis, and substantial amount of work with different model compounds from different research groups, we are is still far from understanding the structure, redox-chemistry and mode of action of this Mn_4-cluster.

It was an intense week of excellent lectures presenting fascinating science spanning from biochemistry to material science, from synthetic organic chemistry to bioinorganic chemistry and from methodology to biology. Even though the selection of areas was highly interdisciplinary, it became clear that the skill and ability to design and synthesise new molecules with new properties, be it small natural products, supramolecular entities, transition metal catalysts or biological macromolecules, is the common denominator. With the relaxed atmosphere embedded in this marvellous location, ample time for scientific discussions and the easiness to get to know people both from industry and academia, the Bürgenstock conference indeed deserves the term 'legendary'.

Acknowledgments

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